Biochimica et Biophysica Acta, 1016 (1990) 289–292
Elsevier

BBABIO 40235

BBA Report

Electron transfer from the reaction center of *Rb. sphaeroides* to the quinone pool: doubly reduced Q_B leaves the reaction center

P.H. McPherson, M.Y. Okamura and G. Feher

Department of Physics, University of California, San Diego, La Jolla, CA (U.S.A.)

(Received 22 November 1989)

Key words: Electron transfer; Proton transfer; Reaction center; Photosynthesis, bacterial; Quinone pool; Chemiosmotic hypothesis; (*Rb. sphaeroides*)

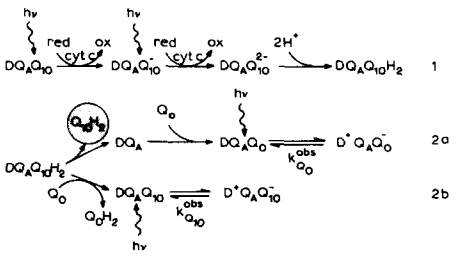
We have tested the hypothesis that electron transfer from the reaction center (RC) to the quinone pool occurs through the release of the doubly reduced secondary quinone Q_B. Doubly reduced Q_B was formed in RCs containing Q_{10} (ubiquinone-50) in the Q_B site in the presence of a pool consisting of Q_0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone). Subsequent measurement of the charge recombination kinetics ($D^+Q_AQ_B^- \rightarrow DQ_AQ_B$) showed a rate characteristic of RCs containing Q_0 in the Q_B site. This proves that the doubly reduced Q_B left the RC and was replaced with a quinone from the pool.

The primary photochemistry in bacterial reaction centers (RCs) involves the light-induced electron transfer from a primary electron donor D (bacteriochlorophyll dimer) through intermediate (ϕ_A, bacteriopheophytin) and primary (Q_A, ubiquinone-50 (Q_{10})) electron acceptors to the secondary acceptor quinone Q_B (Q_{10}) (reviewed in Ref. 1). In the presence of an exogenous donor (i.e., cytochrome c_2^{2+}), the absorption of two photons leads to the formation of $DQ_AQ_B^{2-}$, which protonates, forming $DQ_AQ_BH_2$. These are the initial steps in a series of reactions leading to the chemiosmotic gradient that drives ATP synthesis (reviewed in Ref. 2). In this work, we focus on the subsequent step, in which electrons and concomitantly protons are transferred from $DQ_AQ_BH_2$ in the RC to the quinone (Q_{10}) pool. A preliminary account of this work has been presented [3].

The electron and proton transfer to the quinone pool is believed to occur through the release of the quinol Q_BH_2 from the RC. However, there is no direct experimental evidence of this release. Supporting evidence is provided by the observation that double reduction of Q_B facilitates its removal from the RC [4]. Similar conclusions were reached by Wraight [5] and Diner et al. [6], who found that the order of binding strengths is $Q_B^- > Q_B > Q_BH_2$. Additional support is provided by studies on herbicides such as *ortho*-phenanthroline which work by competing with Q_B for the binding site [4,6]. However, an alternative mechanism proposed earlier [7,8], in which 2 electrons are transferred from Q_B to an exogenous quinone, cannot be strictly ruled out by the published experimental evidence.

We have investigated the problem of quinone exchange by using for the pool a quinone, Q_0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone), that in the RC exhibits different charge recombination kinetics than the native quinone, Q_{10}. Thus, the recombination kinetics provided an assay to determine whether $Q_{10}H_2$ had been exchanged with Q_0.

The method is illustrated in a simplified form * by Eqns. 1 and 2.



Abbreviations: D, primary donor; Q_A, primary acceptor; Q_B, secondary acceptor; RC, reaction center; Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; Q_{10}, ubiquinone-50; LDAO, lauryldimethylamine *N*-oxide; Pipes, 1,4-piperazinediethanesulfonic acid.

Correspondence: G. Feher, Department of Physics, B-019, University of California at San Diego, La Jolla, CA 92093, U.S.A.

* See footnote, p. 290.

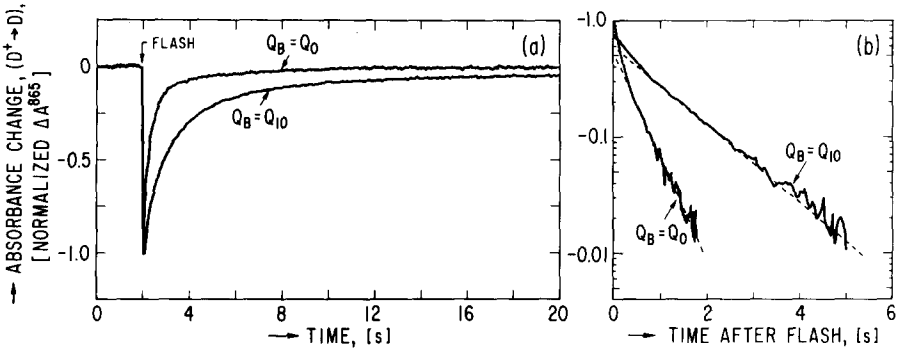


Fig. 1. Determination of the charge recombination rates $k_{Q_0}^{obs}$ ($D^+Q_AQ_0^- \rightarrow DQ_AQ_0$) and $k_{Q_{10}}^{obs}$ ($D^+Q_AQ_{10}^- \rightarrow DQ_AQ_{10}$). (a) Change in absorbance at 865 nm (normalized) due to charge separation and recombination. In the upper trace the Q_B site was occupied by Q_0 and in the lower trace by Q_{10}. Conditions: 5 mM Tris-HCl, 5 mM Pipes, 50 mM KCl, 0.025% LDAO, pH 7.5, $T = 23°$; Upper trace: 2.0 μM RCs (0.8 Q_{10}/RC), 4 μM oxidized cytochrome c_2, 100 μM Q_0; Lower trace: 1.9 μM RCS (2 Q_{10}/RC), 4 μM oxidized cytochrome c_2, 20 μM Q_{10}. (b) Semilog plots of data in (a) with slow phases amounting to 10–20% subtracted for simplicity. Dashed lines are fits to the majority phases of the kinetics which corresponds to $k_{Q_0}^{obs}$ (lower trace) and $k_{Q_{10}}^{obs}$ (upper trace) (see Eqns. 3 and 4).

Initially the RC has the native quinone, Q_{10}, in the Q_B site. A pool of exogenous Q_0 is added together with 2 reduced cytochrome c_2 per RC. After two laser flashes, the state $DQ_AQ_{10}H_2$ is formed (Eqn. 1); in this process the two cytochromes are oxidized.

The two possible mechanisms for electron and proton delivery to the pool are illustrated by Eqns. 2a and 2b. In the scheme represented by Eqn. 2a, $Q_{10}H_2$ leaves the RC and is replaced by Q_0. In the scheme shown by Eqn. 2b, Q_{10} remains in the Q_B site and the two electrons and two protons are taken up by Q_0 in the pool. A third laser flash produces a charge separation between D and Q_B. The charge recombination rate between D^+ and Q_B^- depends on whether the Q_B site is occupied by Q_0 or Q_{10}. This rate was measured spectroscopically at 865 nm and used to differentiate between the two schemes.

RCs [11] and cytochrome c_2 [12] were isolated from *Rb. sphaeroides* as described. The concentration of RCs was determined from the absorbance at 802 nm and the extinction coefficient $\epsilon^{802} = 288\ mM^{-1} \cdot cm^{-1}$ [13] and of cytochrome c_2^{2+} at 550 nm and $\epsilon_{reduced}^{550} = 27.6\ mM^{-1} \cdot cm^{-1}$ [14]. RCs contained 1.92 ± 0.04 Q_{10} per RC (determined from the average of a cytochrome photooxidation and a charge recombination assay [15]). RCs with 0.8 Q_{10}/RC were prepared as described [4]. For experiments with a Q_{10} pool, excess exogenous Q_{10} in 10% (w/v) sodium deoxycholate was added to RCs ($\approx$ 10 Q_{10}/RC; after dilution, [sodium deoxycholate] = 0.5%) followed by extensive dialysis against 10 mM Tris-HCl/0.025% (w/v) LDAO/0.1 mM EDTA (pH 8). Charge recombination rates were measured spectroscopically at 865 nm using a single-beam spectrophotometer of local design [9]. Their values (i.e., Eqns. 3–6) were determined by averaging the results of 4–9 experiments; errors represent the standard deviations of the means.

Since the method illustrated in Eqn. 2 is based on the difference between $k_{Q_0}^{obs}$ and $k_{Q_{10}}^{obs}$, these two quantities had to be determined first. The state $D^+Q_AQ_0^-$ was formed by a single laser flash in RCs with 0.8 Q_{10}/RC (i.e., Q_{10} only in the Q_A site) in the presence of a Q_0 pool ($[Q_0] = 100\ \mu M$). The absorbance changes at 865 nm (due to charge separation and recombination) are shown in Fig. 1a. For comparison, the absorbance change in RCs that had Q_{10} in the Q_B site is also shown (Fig. 1a). As can be seen, there is a significant difference between $k_{Q_0}^{obs}$ and $k_{Q_{10}}^{obs}$.

To obtain the values of the recombination rates, the data of Fig. 1a were replotted semilogarithmically with a slow phase ($k \leq 0.2\ s^{-1}$ amounting to 10–20% sub-

* Several details have been omitted in Eqns. 1 and 2. Although none of them affects the conclusions, they are important in understanding the results quantitatively and are, therefore, listed here: (i) After the first flash, $DQ_A^-Q_{10}$ is in equilibrium with $DQ_AQ_{10}^-$; about 10% of the RCs are in the state $DQ_A^-Q_{10}$ [9]. After the second flash they reequilibrate with $DQ_AQ_{10}^-$ so that about 10% of the RCs are in the state $D^+Q_AQ_{10}^{2-}$ after the third flash. (ii) In scheme 2a, Q_0 is shown for simplicity to be bound prior to charge separation. In reality, Q_0 is thought to bind after charge separation: $D^+Q_A^- + Q_0 \rightarrow D^+Q_A^-Q_0 \rightarrow D^+Q_AQ_0^-$. (iii) In RCs depleted of Q_{10} (e.g., after $Q_{10}H_2$ leaves), Q_0 at a concentration of 100 μM binds and forms $D^+Q_AQ_0^-$ in only about 50% of the RCs. A higher concentration of Q_0 would increase the bound fraction, but would also result in a slower $k_{Q_0}^{obs}$ [10]. The lower Q_0 concentration (100 μM) was chosen to maximize the difference between $k_{Q_0}^{obs}$ and $k_{Q_{10}}^{obs}$ (discussed below). (iv) The reduction of D^+ by cytochrome c_2^{2+} is not complete, because of the finite difference between the redox midpoint potentials of cytochrome c_2^{2+}/cytochrome c_2^{3+} and D/D^+ ($\Delta E \cong 100$ mV). Almost complete (95%) reduction of D^+ was obtained by adding slightly more than 2 cytochrome c_2^{2+} per RC. The fraction (5%) not reduced after the second flash were in the state $D^+Q_AQ_{10}^{2-}$ after the third flash. (v) Oxidized cytochrome c_2 (present as a consequence of the reduction of D^+) caused the appearance of a slow phase ($k \leq 0.2\ s^{-1}$) in the charge recombination kinetics amounting to 10–20% of the total amplitude. This may be caused by the bound oxidized cytochrome, which could change the protein structure near D^+.

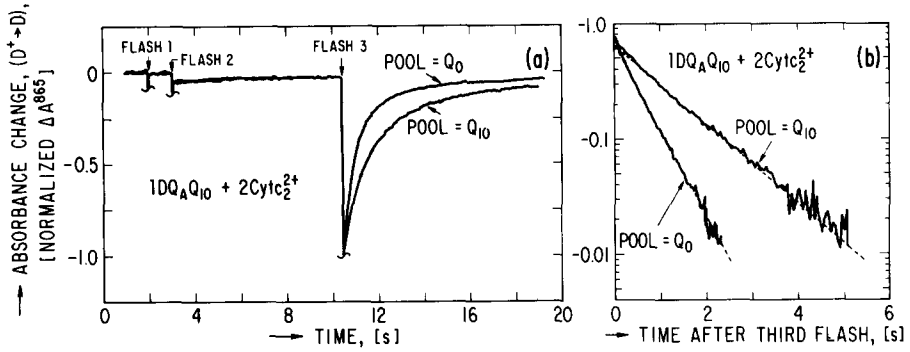


Fig. 2. Charge recombination assay to determine whether Q_BH_2 leaves the RC (see Eqns 2a, 2b). (a) Change in absorbance at 865 nm following three laser flashes in RCs starting with Q_{10} in the Q_B site in the presence of two reduced cytochrome c_2 and either a Q_0 pool (upper trace) or a Q_{10} pool (lower trace). Spikes after flashes are due to fast (< 20 ms) reduction of D^+ by cytochrome c_2^{2+}. Conditions: 5 mM Tris-HCl, 5 mM Pipes, 50 mM KCl, 0.025% LDAO (pH 7.5) $T = 23°C$; Upper trace: 2.4 μM RCs (1.92 ± 0.04 Q_{10}/RC), 5 μM cytochrome c_2^{2+}, 100 μM Q_0; Lower trace: 2.3 μM RCs, 5 μM cytochrome c_2^{2+}, 20 μM Q_{10}. (b) Semilog plots of data in (a) with slow phases amounting to about 25% subtracted for simplicity. Dashed lines are fits to the majority phases of the kinetics which correspond to charge recombination between D^+ and Q_B^-. In lower trace, the rate (see Eqn. 5) corresponds to $k_{Q_0}^{obs}$ (see Eqn. 3) proving that $Q_{10}H_2$ left the RC and was replaced by Q_0.

tracted for simplicity (Fig. 1b). The slow phase was due mainly to the presence of oxidized cytochrome c_2, which was added to simulate the conditions of the charge recombination assay (see Eqns. 1, 2 and footnote *). The dashed lines in Fig. 1b represent straight line fits to the majority phase of the kinetics, which were interpreted as being due to the recombination rates $k_{Q_0}^{obs}$ ($D^+Q_AQ_0^- \rightarrow DQ_AQ_0$) and $k_{Q_{10}}^{obs}$ ($D^+Q_AQ_{10}^- \rightarrow DQ_AQ_{10}$), respectively. Their values are:

$$k_{Q_0}^{obs} = 1.7 \pm 0.2\ s^{-1}\ ([Q_0] = 100\ \mu M) \qquad (3)$$

$$k_{Q_{10}}^{obs} = 0.84 \pm 0.04\ s^{-1} \qquad (4)$$

These values are in agreement with those reported for $k_{Q_0}^{obs}$ [10] and $k_{Q_{10}}^{obs}$ [9].

At short times the dashed lines deviate from the experimentally determined kinetics. This is attributed mainly to the charge recombination $D^+Q_A^- \rightarrow DQ_A$ in the fraction of RCs that lacked Q_B (see footnote *).

We now proceed to discuss whether scheme 2a or 2b holds. As discussed above, the RCs started with Q_{10} in the Q_B site in the presence of a Q_0 pool and 2 cytochrome c_2^{2+} per RC. The absorbance changes at 865 nm (due to the formation and reduction of D^+) following three laser flashes are shown in Fig. 2a. After the first and second flashes the states $DQ_AQ_0^{-1}$ and $DQ_AQ_{10}H_2$ were formed. The cytochrome c_2^{2+} reduced D^+ faster than the time constant of the apparatus (20 ms); consequently, only small absorbance changes were observed after the first two flashes (see Fig. 2a). After the second flash, most of the cytochrome had been oxidized (see footnote *). Following the third flash, charge separation and recombination could be observed.

The recombination kinetics with the slowest phases subtracted are shown in Fig. 2b. The slowest phases were due in part to the approx. 15% admixture of the state $D^+Q_AQ_{10}^{2-}$ and in part to the effect of the oxidized cytochrome c_2 (see footnote *). The majority phase (see dashed straight line in Fig. 2b) is due to charge recombination between D^+ and Q_B^-; its rate is:

$$Q_0 \text{ pool: } k = 1.9 \pm 0.2\ s^{-1} \cong k_{Q_0}^{obs}\ ([Q_0] = 100\ \mu M). \qquad (5)$$

This rate agrees within experimental error with $k_{Q_0}^{obs}$ (see Eqn. 3), proving that Q_BH_2 is released from the RC as described in Eqn. 2a.

The experiment was repeated with a Q_{10} pool (Fig. 2). In this case, either scheme (2a or 2b) results in the recombination rate $k_{Q_{10}}^{obs}$. The observed rate is:

$$Q_{10} \text{ pool: } k = 0.82 \pm 0.02\ s^{-1} = k_{Q_{10}}^{obs}, \qquad (6)$$

in good agreement with the value given in Eqn. 4.

In Eqns. 1 and 2 we have assumed that neither the quinone Q_{10} nor the semiquinone Q_{10}^- is displaced by Q_0. This was checked by measuring the charge recombination kinetics after a single flash in RCs that started with Q_{10} in the Q_B site in the presence of Q_0 pool ($[Q_0] = 100\ \mu M$). The charge recombination rate corresponded to $k_{Q_{10}}^{obs}$, showing that neither Q_{10} nor Q_{10}^- was displaced by Q_0.

In summary, we have shown that the doubly charged Q_B^{2-} (most likely in the quinol form Q_BH_2) is released from the RC and replaced with a quinone Q from the pool. This process is the final step in the photochemical cycle of the reaction center, i.e., it returns the RC to the starting state DQ_AQ_B.

We thank Ed. Abresch for preparing the reaction centers and K. Giangiacomo and P.L. Dutton for helpful discussions on different quinones in the Q_B site. The work was supported by a grant from the National Science Foundation (DMB 85-18922).

References

1 Parson, W.W. (1987) in Photosynthesis (Amesz, J., ed.), pp. 43–61, Elsevier, New York.

2 Dutton, P.L. (1986) in Encyclopedia of Plant Physiology, Vol. 19: Photosynthesis III (Staehelin, L.A., and Arntzen, C.J., eds.), pp. 197–237, Springer, New york.

3 Feher, G., McPherson, P.H., Paddock, M., Rongey, S.H., Schonfeld, M. and Okamura, M.Y. (1989) in Proceedings of the VIIIth International Congress on Photosynthesis, in press.

4 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3491–3495.

5 Wraight, C.A. (1981) Isr. J. Chem 21, 348–354.

6 Diner, B.A., Schenck, C.C. and De Vitry, C. (1984) Biochim. Biophys. Acta 776, 9–20.

7 Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524.

8 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531.

9 Kleinfeld, D., Okamura, M.Y. and Feher, G. (1984) Biochim. Biophys. Acta 766, 126–140.

10 Wraight, C.A. and Stein, R.E. (1983) in The Oxygen Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 383–392, Academic Press, Japan.

11 Feher, G., and Okamura, M.Y. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 349–386, Plenum, New York.

12 Bartsch, R.G. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 249–279, Plenum, New York.

13 Straley, S.C., Parson, W.W., Mauzerall, D.C. and Clayton, R.K. (1973) Biochim. Biophys. Acta 305, 597–609.

14 Margoliash, E. and Frohwirt, N. (1959) Biochem. J. 71 570–572.

15 Okamura, M.Y., Debus, R.J., Kleinfeld, D., and Feher, G., (1982) in Function of Quinones in Energy-Conserving Systems (Trumpower, B.L., ed.), pp. 299–317, Academic Press, New York.